

1 BIOLOGICAL MATERIAL DETECTING ARTICLES OF MANUFACTURE

2
3 CROSS-REFERENCE TO RELATED APPLICATIONS

4 This application is a continuation-in-part of
5 S.N.09/724,438, filed November 28, 2000, which is a
6 continuation-in-part of copending applications S.N.
7 09/555,777, filed on April 17, 2000 and 09/555,779, filed on
8 April 17, 2000 now U.S. Patents _____ and
9 _____ respectively; which are continuations-in-part of
10 application S.N. 09/218,827, filed December 22, 1998, now
11 U.S. Patent 6,051,388, having an issue date of April 18,
12 2000; all of the contents of which are herein incorporated by
13 reference.

14
15 FIELD OF THE INVENTION

16 This invention relates to articles of manufacture
17 comprising a biological assay material for detecting the
18 presence of a particular toxic substance; particularly to
19 articles of manufacture comprising active areas which are
20 constructed and arranged for the diagnostic detection and
21 identification of pathological agents; and most particularly
22 to articles of manufacture particularly designed for
23 detecting and identifying one or a plurality of materials
24 which are biologically hazardous.

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4 BACKGROUND OF THE INVENTION

5 Although considerable effort and expense have been put
6 forth in an effort to control food and/or airborne pathogenic
7 microorganisms, there nevertheless exist significant safety
8 problems in the supply of packaged food, in the certification
9 of sterility for medically useful components, e.g. surgical
10 tools, internal examination devices, e.g. endoscopes, and the
11 like, and in dealing with the use of a variety of biological
12 materials as weapons of mass destruction.

13 For example, numerous outbreaks of food poisoning
14 brought about by foodstuffs contaminated with strains of the
15 E-Coli, Campylobacter, Listeria, Cyclospora and Salmonella
16 microorganisms have caused illness and even death, not to
17 mention a tremendous loss of revenue for food producers.
18 These and other microorganisms can inadvertently taint food,
19 even when reasonably careful food handling procedures are
20 followed. The possibility of accidental contamination, for
21 example by temperature abuse, in and of itself, is enough to
22 warrant incorporation of safe and effective biological
23 material diagnosis and detection procedures. Further
24 complicating the situation is the very real possibility that

1 a terrorist organization might target either the food or
2 water supply of a municipality or even a nation itself, by
3 attempting to include a pathogenic microorganism or toxic
4 contaminant capable of causing widespread illness or even
5 death. If, by accident or design, the food supply of a
6 particular population were to be contaminated, it is not only
7 imperative that the population be alerted to the
8 contamination, but it is further necessary that the
9 particular contaminant be quickly and precisely pinpointed so
10 that appropriate countermeasures may be taken.

11 With respect to medical or dental procedures, there
12 exists a very real possibility for transmission of disease
13 due to ineffective sterilization techniques or careless
14 handling of medical implements, which can often lead to
15 contamination of the sterile field. Although these devices
16 are generally wrapped after sterilization, it is impossible
17 to verify the efficacy of the sterilizing process or
18 determine if subsequent contamination has occurred prior to
19 use.

20 Additional attention is directed toward the use of
21 potential agents of bioterrorism, e.g. various bacteria,
22 viruses, or toxins, which can be of microbial, plant, or
23 animal origin, also represent a credible threat to the
24 general population, since they can be incorporated within

1 biological weapon systems of mass destruction.. The most
2 common agents of concern include *Bacillus anthracis*
3 (anthrax), *Yersinia pestis* (plague), Variola major virus
4 (smallpox), and botulinum toxin. Additional potential agents
5 include: brucella sp.; Venezuelan equine encephalitis (VEE)
6 virus and other viral encephalidities; *Vibrio cholerae*
7 (cholera); and, staphylococcal enterotoxin B (SEB).

8 The technology required to creates weapons of mass
9 destruction from biological agents is readily available to
10 the civilian population in the form of texts and information
11 available via the Internet. Modestly financed organizations
12 of relatively small size and rather basic training
13 in biology and engineering could easily develop an effective
14 biological weapons capability.

15 Individual agents and toxins useful as biological
16 weapons generally share the following features: (1)
17 capability of being dispersed as aerosols and remain
18 suspended for hours; (2) aerosols are deliverable by
19 simple technology readily available in industry, e.g.,
20 agricultural crop dusters, backpack sprayers, purse-size
21 perfume atomizers, and the like; and, (3) aerosols are
22 capable of producing significant, if not fatal, illness in
23 humans when inhaled.

24 In contrast to screening methods used to detect

1 traditional explosive devices (e.g., x-ray and trained
2 canines), there are essentially no routine methods or
3 technology in place to detect a biological weapon.
4 Additionally, variously known laboratory techniques for
5 detecting biological agents require extensive time for
6 development and testing of sample cultures in order to
7 confirm a diagnosis.

8 Lastly, it is generally accepted that it is impossible
9 to know either the timing for release of a biological agent
10 or the methodology of its dispersal, e.g. aerosol, powder,
11 via the mails, through HVAC systems, or the like.

12 Thus, it is imperative that articles of manufacture be
13 developed which provide an unambiguous warning to the
14 untrained general population, that they have come in contact
15 with a biological weapon.

16 DESCRIPTION OF THE PRIOR ART

17 U.S. Patent 6,051,388 discloses bioassay materials which
18 may take the form of packaging materials for food or other
19 products and which are useful for detecting toxic substances
20 The biological assay therein disclosed broadly encompasses a
21 base layer which is a flexible polyolefin film having a
22 surface which has undergone a treatment step effective to
23 enhance the film's ability to immobilize a ligand which has
24 been applied thereto and a biologically active ligand which

1 is immobilized to the film subsequent to which a protectant
2 layer in the form of a gel coat or liquid film is applied.
3 This patent requires separate deposition of the active ligand
4 followed by application of the protectant layer.

5 U.S. Patent No. 4,966,856 discloses an analytical
6 element having a layer for antibody/antigen binding but fails
7 to teach or suggest a flexible polyolefin matrix.

8 U.S. Patent No. 4,870,005 teaches a multi-layer analysis
9 element including a membrane filter to which an antigen or
10 antibody is immobilized. The concept of forming a flexible
11 analysis element having immobilized biological agents bound
12 thereto is neither suggested nor disclosed.

13 U.S. Patent No. 6,020,047 discloses a polymer film
14 coated with a metal alloy and containing a self-assembling
15 monolayer printed on the polymer film.

16 U.S. Patent 5,898,373 discloses a method for monitoring
17 a site for the presence of future toxic agents. The patent
18 places sticky polymeric particles upon a site to be remotely
19 monitored for toxins over a future time period. Upon contact
20 with a toxic agent, the particles react to produce or reflect
21 a particular spectral signature which may be verified via an
22 airborne vehicle using a laser transmitter or the like
23 investigative tool.

24 U.S. Patent 5,614,375 teaches a method and a test kit

1 for rapidly detecting biotoxic contaminants. Activated
2 spores, devoid of enzymatic activity, are germinated and
3 enzymatic activity is determined in the presence of a
4 material which is catalytically convertible to a product by
5 the enzymatic activity. Conversion of the material is
6 determined as a means of verifying the presence of the toxic
7 material.

8 The Berkeley Lab Research News of 12/10/96, in an
9 article entitle "New Sensor Provides First Instant Test for
10 Toxic E.Coli Organism" reports on the work of Stevens and
11 Cheng to develop sensors capable of detecting E. Coli strain
12 0157:H7. A color change from blue to red instantaneously
13 signals the presence of the virulent E. Coli 0157:H7
14 microorganism. Prior art required test sampling and a 24
15 hour culture period in order to determine the presence of the
16 E. Coli microorganism, requiring the use of a variety of
17 diagnostic tools including dyes and microscopes. An
18 alternative technique, involving the use of polymerase chain
19 reaction technology, multiplies the amount of DNA present in
20 a sample until it reaches a detectable level. This test
21 requires several hours before results can be obtained. The
22 Berkeley sensor is inexpensive and may be placed on a variety
23 of materials such as plastic, paper, or glass, e.g. within a
24 bottle cap or container lid. Multiple copies of a single

1 molecule are fabricated into a thin film which has a two part
2 composite structure. The surface binds the biological
3 material while the backbone underlying the surface is the
4 color-changing signaling system.

5 The Berkeley researchers do not teach the concept of
6 incorporating any means for self-detection within food
7 packaging, nor do they contemplate the inclusion of multiple
8 means capable of both detecting and identifying the source of
9 pathogenic contamination to a technically untrained end user,
10 e.g. the food purchaser or consumer.

11 Wang et al, in an article entitled "An immune-capturing
12 and concentrating procedure for Escherichia coli 0157:H7 and
13 its detection by epifluorescence microscopy" published in
14 Food Microbiology, 1998, Vol. 15 discloses the capture of E.
15 coli on a polyvinylchloride sheet coated with polyclonal
16 anti-E. coli 0157:H7 antibody and stained with fluorescein-
17 labeled anti-E. coli 0157:H7. After being scraped from the
18 PVC surface, the cells were subjected to epifluorescence
19 microscopy for determining presence and concentration. The
20 reference fails to teach or suggest the concept of
21 incorporating any means for self-detection within food
22 packaging, nor does it contemplate the inclusion of multiple
23 means capable of both detecting and identifying the source of
24 pathogenic contamination to a technically untrained end user,

1 e.g. the food purchaser or consumer, and especially fails to
2 disclose such detection without the use of specialized
3 detection techniques and equipment.

4 U.S. Patent 5,776,672 discloses a single stranded
5 nucleic acid probe having a base sequence complementary to
6 the gene to be detected which is immobilized onto the surface
7 of an optical fiber and then reacted with the gene sample
8 denatured to a single stranded form. The nucleic acid probe,
9 hybridized with the gene is detected by electrochemical or
10 optical detection methodology. In contrast to the instantly
11 disclosed invention, this reference does not suggest the
12 immobilization of the probe onto a flexible polyvinylchloride
13 or polyolefin film, nor does it suggest the utilization of
14 gelcoats having varying porosities to act as a control or
15 limiting agent with respect to the migration of antibodies or
16 microbial material through the bioassay test material, or to
17 serve as a medium for enhancement of the growth of the
18 microbial material.

19 U.S. Patent 5,756,291 discloses a method of identifying
20 oligomer sequences. The method generates aptamers which are
21 capable of binding to serum factors and all surface
22 molecules. Complexation of the target molecules with a
23 mixture of nucleotides occurs under conditions wherein a
24 complex is formed with the specific binding sequences but not

1 with the other members of the oligonucleotide mixture. The
2 reference fails to suggest the immobilization of the aptamers
3 upon a flexible polyvinylchloride or polyolefin base
4 material, nor does it suggest the use of a protective gelcoat
5 layer which acts as a means to selectively control the
6 migration of antibodies and antigens, or to serve as a medium
7 for enhancement of the growth of microbial material.

8 The prior art fails to teach an article of manufacture
9 which is readily providable to the populous, and which can
10 provide an unskilled person with a visual signal capable of
11 alerting said individual to the presence of a toxic agent
12 while simultaneously identifying the toxic agent with which
13 the individual has come into contact.

14 15 SUMMARY OF THE INVENTION

16 The present invention relates to articles of manufacture
17 inclusive of or in combination with a biological assay
18 material, wherein "in combination" may be defined as integral
19 therewith, or appended thereto or thereon. The articles of
20 the instant invention are formed a material capable of
21 detecting and identifying a multiplicity of biological
22 materials.

23 In one embodiment, the article of manufacture, which is
24 contemplated as including various articles of clothing (non-

1 limiting examples of which are gloves, lab-coats, booties,
2 hats, face masks, and the like) labels, envelopes, bags or
3 pouches, self-adherent patches, and the like; are formed so
4 as to provide an integral biological material identification
5 system. By "integral" it is meant that the biological
6 material detection system may constitute the material of
7 construction of the biological assay material, may be applied
8 directly to the article of manufacture, or alternatively,
9 said article may be constructed and arranged to accept a
10 portion of said biological material detection system thereon,
11 in an amount effective to provide the desired indication of
12 contamination. In such an embodiment, the biological
13 material detection system is designed to be easily replaced
14 so that the base article is instantly reusable upon
15 application of a new or different biological assay material.
16 Thus, using gloves as an illustrative embodiment, such gloves
17 could be formed for extended use, while the biological assay
18 material could be easily rejuvenated or changed, so as to
19 facilitate maintenance of the diagnostic efficacy of the
20 gloves or alternatively to enable instantaneous customization
21 of the gloves for a particular detection utility. Given the
22 varying means by which the biological material detecting
23 system of the instant invention can be included in
24 combination with various articles of manufacture, the

1 widespread inclusion of the biological material detecting
2 system in a variety of manufactured articles will be both
3 efficient and economical.

4 In one embodiment of the invention the biological
5 material detecting system prints a pattern containing several
6 of the biologically active agents, e.g. antibodies or
7 aptamers onto a flexible material which is usually a type of
8 polymeric film, preferably a polyvinyl chloride or polyolefin
9 film.

10 Each biological agent, for example an antibody, can be
11 tailored so as to be specific to a particular biological
12 material and may be printed upon the substrate in a
13 distinctive icon shape. The detection system may contain any
14 number of biological agents, or a variety of epitopes
15 thereof, capable of detecting a variety of common toxic
16 microbes, less common microbes useful as biological weapons,
17 or combinations thereof. Although any number of microbes may
18 be identified via the inventive concept taught herein, for
19 the purpose of this description, the microbes of interest
20 will be directed toward Anthrax, Smallpox, Plague and
21 Botulism.

22 The biological material detecting system will not merely
23 detect the presence of biological materials, it will also
24 identify the particular biological materials located in a

1 packaged product. This unique feature allows for the
2 immediate identification of each particular biological
3 material present since the antibodies are specific to a
4 detector having a definitive icon shape or other identifying
5 characteristic. As an illustrative, but non-limiting
6 embodiment of the invention, a plurality of icons, each
7 relevant to a particular biohazard, e.g. Anthrax, Smallpox,
8 Plague, Botulism and the like, can be applied to the
9 substrate via various printing techniques, as are set forth
10 in U.S. Patent 6,051,388 and related applications, all of
11 whose contents have been herein incorporated by reference.
12 Upon contact with one or more of the biohazards, the icons
13 will change from their original visual image to an image
14 which is indicative of said contact, thereby alerting the
15 viewer of a dangerous situation, while simultaneously
16 identifying the biohazard.

17 The ability to detect and identify the particular
18 biological material immediately is of immeasurable value to
19 health officials and governmental agencies. The ability to
20 immediately identify a toxic material will lead to greatly
21 reduced response times to health threats that might be caused
22 by the biological material and will also enhance the ability
23 for authorities to locate the source of the problem.

24 In an alternative embodiment, the biological material

1 detection system may be formed upon any suitable substrate
2 e.g. any flexible transparent polymer film, and subsequently
3 be combined with a secondary material, illustrated, but not
4 limited to, a paper or cloth backing, which may further
5 contain means for adherence to yet an additional article. In
6 such manner, an article of manufacture useful for producing
7 an unlimited variety of end-products is contemplated by the
8 invention.

9 As a means of providing enhanced sensitization, a
10 scavenger antibody, which is a biologically active ligand
11 characterized as having a higher affinity for the particular
12 toxic substance than the capture antibody, may be included.
13 The scavenger antibody is provided, e.g. by mixing said
14 scavenger body with the combined capture antibody/water gloss
15 overprint varnish, in a sufficient amount to bind with the
16 particular toxic substance up to and including a specific
17 threshold concentration. In this manner, the capture
18 antibody will be prevented from binding with a detector
19 antibody until the concentration of the particular biological
20 material surpasses the specific threshold concentration. In
21 this manner, the biological material detecting system
22 visually reports only those instances where concentration
23 levels are deemed harmful by health regulatory bodies.

24 The biological material detecting system of the present

1 invention exhibits an active shelf life in excess of 1 year
2 under normal operating conditions. This enhances the use of
3 a biological material detection system on products which are
4 intended to be stored for long periods of time, e.g. military
5 rations or medical supplies, which might come into contact
6 with biological hazards. These products are stored so as to
7 be ready for immediate use in some time of emergency,
8 therefore it is extremely beneficial to be able to readily
9 determine their safety at the time of use.

10 The articles of manufacture which incorporate the
11 biological material detecting system, as set forth in the
12 instant invention, represent an entirely new device for
13 alerting the general population to the presence of toxic
14 materials in the environment. They provide the layman with a
15 simple device, which is easily substituted for non-
16 biologically sensitive devices, which will readily alert
17 users to the presence of certain biologically hazardous
18 materials present in food stuffs, mail, newspapers, or the
19 like.

20 The system is designed so that the presence of a
21 biological material is indicated to the user in a distinct,
22 unmistakable manner which is easily visible to the naked eye.

23 An important feature of the biological material
24 detection system is the plurality of testing sites which it

1 provides. In the past, the use of single location or *in situ*
2 detectors have left a majority of the area around and upon a
3 particular location exposed to undetected microbes. This
4 greatly increased the chance that a hazardous, spoiled or
5 tainted product might be inadvertently passed along or
6 consumed before the toxic agent had spread to the location of
7 the *in situ* detector. The biological material detection
8 system of the present invention avoids this problem by
9 providing a plurality of individual detectors per unit area
10 which are effective to maximize detection of any hazardous
11 microorganisms within, upon or around the area of concern.

12 It is an objective of the present invention to provide
13 an article of manufacture which comprises a biological
14 material detecting system for protecting against, or warning
15 of the presence of, a biologically hazardous material.
16 Awareness of the hazardous material is accomplished by
17 detecting and unmistakably presenting to the untrained eye
18 visual icons on said article which signify the presence of
19 one, or a plurality, of hazardous microorganisms.

20 It is another objective of the instant invention to
21 provide an article of manufacture which integrates a bioassay
22 material detection system, wherein an antigen detecting
23 antibody system is immobilized within a biological activity
24 maintaining matrix (e.g. a gelcoat layer and/or a varnish

1 matrix) upon the surface of a flexible polymer.

2 It is still another objective of the instant invention
3 to provide an article of manufacture comprising a bioassay
4 material wherein an antigen detecting antibody system is
5 immobilized upon the surface of a suitable substrate, e.g. a
6 flexible member formed from a polymer film, or a composite
7 laminated structure including said film.

8 It is a further objective of the invention to provide an
9 article of manufacture inclusive of a biological material
10 detecting system which is so similar in appearance and
11 utilization that its use, in lieu of traditional articles of
12 manufacture, is not apparent to the end user.

13 A still further objective of the present invention is to
14 provide an article of manufacture inclusive of a biological
15 material detecting system which is cost effective when
16 compared to traditional packaging materials.

17 Yet an additional objective of the instant invention is
18 to provide an article of manufacture inclusive of a
19 biological material detecting system applied to a substrate.

20 Other objects and advantages of this invention will
21 become apparent from the following description taken in
22 conjunction with the accompanying drawings wherein are set
23 forth, by way of illustration and example, certain
24 embodiments of this invention. The drawings constitute a

1 part of this specification and include exemplary embodiments
2 of the present invention and illustrate various objects and
3 features thereof.

4 5 DETAILED DESCRIPTION OF THE INVENTION

6 The particular toxic substance may be one or more
7 members selected from the group consisting of a particular
8 microorganism, biological materials containing the genetic
9 characteristics of said particular microorganism, and
10 mutations thereof. In a particular embodiment, the toxic
11 substance is selected from the group consisting of
12 microorganisms, nucleic acids, proteins, integral components
13 of microorganisms and combinations thereof.

14 It should also be understood that the invention will
15 function by direct measurement of microbes with certain types
16 of antibodies, selected from the group consisting of an
17 antibody, a single stranded nucleic acid probe, an aptamer, a
18 lipid, a natural receptor, a lectin, a carbohydrate and a
19 protein. The biological materials may also be measured by
20 non-immunological methods in particular using labeled
21 molecules, such as aptamers, which have a high affinity for
22 the biological materials.

23 The invention utilizes various types of detector
24 antibodies, e.g. those conjugated with dyes to produce a

1 visual cue, or alternatively, photoactive compounds capable
2 of producing a visual cue in response to a particular type of
3 light exposure, for example a scanning system which detects
4 luminescent properties which are visualized upon binding of
5 the antigen and antibody. In this method of construction
6 biological materials are measured directly with a
7 biologically active ligand, e.g. an antibody, aptamer,
8 nucleic acid probe or the like, which induces a
9 conformational change to produce a visual cue.

10 It is also understood that specific polymers may be
11 incorporated into the invention and that when a biological
12 material is bound to the surface it induces a molecular
13 change in the polymer resulting in a distinctly colored icon.

14 The inventor has now discovered that it is possible to
15 form composites by attaching biologically active ligands to
16 the surface of various substrates, e.g. flexible cellulosic
17 materials, e.g. paperstock, flexible polymers, flexible spun
18 or woven materials, and the like, for example polyvinyl
19 chloride, TYVEK, various polyolefins either singly or in
20 varying combinations, e.g. a polyolefin sheet having
21 appropriate properties of transparency and flexibility and
22 that the composite functions as a biological sensor or assay
23 material. These films may be untreated polyethylene or
24 polyvinyl chloride films which are amenable to antibody

1 immobilization by various mechanisms, e.g. by adsorption. In
2 a particular embodiment, the films may be first cleaned, e.g.
3 by ultrasonication in an appropriate solvent, and
4 subsequently dried. For example the polymer sheet may be
5 exposed to a fifteen minute ultrasonic treatment in a solvent
6 such as methylene chloride, acetone, distilled water, or the
7 like. In some cases, a series of solvent treatments are
8 performed. Subsequently the film is placed in a desiccating
9 device and dried. Alternatively, these films may be created
10 by first exposing the film to an electron discharge treatment
11 at the surface thereof, then printing with a fluorescing
12 antibody receptor. Subsequently, a drying or heating step
13 may be utilized to treat the film to immobilize the receptor.

14 Additional modifications to polyolefin films may be
15 conducted to create the presence of functional groups, for
16 example a polyethylene sheet may be halogenated by a free
17 radical substitution mechanism, e.g. bromination,
18 chlorosulfonation,, chlorophosphorylation or the like.
19 Furthermore, a halodialkylammonium salt in a sulfuric acid
20 solution may be useful as a halogenating agent when enhanced
21 surface selectivity is desirable.

22 Grafting techniques are also contemplated wherein
23 hydrogen abstraction by transient free radicals or free
24 radical equivalents generated in the vapor or gas phase is

1 conducted. Grafting by various alternative means such as
2 irradiation, various means of surface modification,
3 polyolefin oxidation, acid etching, inclusion of chemical
4 additive compounds to the polymer formulation which have the
5 ability to modify the surface characteristics thereof, or
6 equivalent techniques are all contemplated by this invention.

7 Additionally, the formation of oxygenated surface groups
8 such as hydroxyl, carbonyl and carboxyl groups via a flame
9 treatment surface modification technique is contemplated.

10 Further, functionalization without chain scission by
11 carbene insertion chemistry is also contemplated as a means
12 of polymer modification.

13 Illustrative of the types of commercially available
14 films which might be utilized are polyvinyl chloride films
15 and a straight polyethylene film with electron discharge
16 treatment marketed under the trademark SCLAIR®. The electron
17 discharge treatment, when utilized, renders the film much
18 more susceptible to immobilization of the antibodies on its
19 surface. Additional films which might be utilized are Nylon
20 66 films, for example DARTEK®, a coextrudable adhesive film
21 such as BYNEL® and a blend of BYNEL® with polyethylene film.

22 Articles of manufacture include, but are not limited to
23 protective gloves, booties, hats, face masks, and the like
24 garments or articles in which the artisan is desirous of

1 including a biological material detection and identification
2 ability.

3 Additional articles of manufacture contemplated by the
4 invention include, but are not limited to containers, e.g.
5 document handling containers, such as mailbags, bags, boxes,
6 envelopes, and the like; various signs and/or labels which
7 may be self-adherent to a particular surface, and badges or
8 tags which may be applied or attached to other articles or
9 structures. The assay material may be attached directly to a
10 substrate of choice, or alternatively a flexible substrate
11 which includes the biological assay utility may be included
12 in combination with a base article, to form a composite
13 structure.

14 The invention will be further illustrated by way of the
15 following examples, any of which may be fashioned into any of
16 the contemplated articles:

17 **EXAMPLE 1**

18 **Detection of Antibody on the Surface of a Thin Layer**

19 **Polyvinylchloride Sheet:**

20 Rabbit polyclonal IgG was diluted to a final concentration of
21 2.0 µg/ml in 0.1M carbonate (Na_2CO_3)-bicarbonate (NaHCO_3)
22 buffer, pH 9.6.

23 Using a 2" x 3" grid, 75 µL (150 ng) was applied to a sheet
24 of polyvinylchloride at 1" intervals.

1 The antibody treated polyvinylchloride sheet was dried for
2 1.5 hrs. at a temperature of 37°C.
3 The dried sheet was then washed 3 times with a phosphate
4 buffered saline solution at a ph of 7.4.
5 HRP conjugated goat anti-rabbit IgG ($G\alpha R^{HRP}$) was diluted to a
6 concentration of 1:7000 in 1% casein, 0.1M potassium
7 ferricyanide $K_3Fe(CN)_6$, 0.1% phosphate glass ($Na_{15}P_{13}O_{40}$ -
8 $Na_{20}P_{18}O_{55}$), at a pH of 7.4.
9 A precision pipette was used to apply 125 μ L of diluted G^{HRP}
10 to the grid backed polyvinylchloride sheet at 1" intervals
11 coinciding with the area covered by the previously coupled
12 R α G.
13 The sheet was incubated at room temperature for 30 minutes.
14 The sheet was then washed 3X with phosphate buffered saline
15 at a pH of 7.4.
16 125 μ L of precipitating TMB enzyme substrate was added to the
17 test areas.
18 The sheet was incubated at room temperature until color
19 development was complete.
20 Lastly the sheet was washed 3 times with deionized water and
21 allowed to air dry.

22 **EXAMPLE 2**

23 **Full Sandwich Immunoassay on the Surface of a Thin Layer** 24 **Polyvinylchloride Sheet**

1 Rabbit polyclonal IgG was diluted to a final
2 concentration of 2.0 µg/ml in 0.1M carbonate (Na₂CO₃)-
3 bicarbonate (NaHCO₃) buffer, pH 9.6.

4 A 13 x 9 cm piece of thin layered polyvinylchloride
5 sheet was inserted into a BIO-RAD DOT-SPOT apparatus
6 possessing 96 sample wells spaced at 1.0 cm intervals in a 12
7 x 8 well grid.

8 A 100 µL sample (1.0 µg) of rabbit polyclonal IgG was
9 applied to each well 8 of column 1.

10 Antibody samples applied to columns 2-12 represented
11 serial dilutions of the antibody ranging from 500 ng - 0.5
12 ng.

13 The antibody treated polyvinylchloride sheet was dried
14 overnight at 37° C.

15 The dried sheet was washed 3 times with phosphate
16 buffered saline (PBS), pH 7.4.

17 Antigen was diluted to a final concentration of 1.0
18 µg/ml in tris buffered saline (TBS) with 1% casein, pH 7.4.

19 100 µL, representing 100 ng, of antigen, was applied to
20 each well of the apparatus and incubated at room temperature
21 for 1 hour.

22 The polyvinylchloride sheet was washed 3 times with
23 phosphate buffered saline (PBS), pH 7.4.

24 Detector mouse monoclonal antibody was diluted 1:625

1 with TBS containing 1% casein, 0.1M potassium ferricyanide
2 $K_3Fe(CN)_6$, and 0.1% phosphate glass ($Na_{15}P_{13}O_{40} - Na_{20}P_{18}O_{55}$), pH
3 7.4.

4 100 μ L of the 1:625 dilution of detector antibody
5 solution was applied to each well of row # 1.

6 Detector samples of 100 μ L applied to rows 2-7
7 represented serial dilutions of the antibody ranging from
8 1:1,250 to 1:80,000. Dilutions of detector antibody were
9 incubated on the polyvinylchloride sheet for 1 Hr. at room
10 temperature.

11 The polyvinylchloride sheet was washed 3 times with
12 phosphate buffered saline (PBS), pH 7.4.

13 100 μ L of goat anti-mouse IgG^{HRP} were added to each well
14 of the DOT-SPOT apparatus and allowed to incubate for one
15 hour at room temperature.

16 The polyvinylchloride sheet was washed 3 times with
17 phosphate buffered saline (PBS), pH 7.4.

18 100 μ L of precipitating TMB enzyme substrate was added
19 to the test areas.

20 The sheet was incubated at room temperature until color
21 development was complete.

22 Lastly the sheet was washed 3 times with deionized water
23 and allowed to air dry.

24

EXAMPLE 3

1. Water Gloss FDA Overprint Varnish WVG001006 was diluted with UHF pure water to final concentrations of 1:2, 1:5, 1:10, 1:20, 1:40, and 1:80.

The varnish has the properties of being grease resistant, heat resistant to 175° F, 30 PSI, 2 sec. dwell, Krome Kote, face to paper; COF 25° - 30° F, clear, glossy finish, non-scuff resistant, not imprintable, viscosity/CPS 20-25 sec, #3 Zahn at 77° F, pH 9.2 - 9.6.

2. A monoclonal anti-Listeria monocytogenes capture immunoglobulin (MAb 833) was added to each dilution of the varnish, including one aliquot of neat (undiluted) varnish, for a final concentration of 20 ug/mL in each sample.

3. A sheet of corona discharge treated PE was placed between two pieces of acrylic, of which the uppermost component served as a template. The template possessed 7 columns of 5 bottomless X shaped wells in which samples could be applied directly to the surface of the PE. The two acrylic components were secured by a series of clamps and bolts to prevent leakage.

4. 10 µL of the undiluted varnish, containing 200 ng of immunoglobulin, was applied to each well of column 1. The procedure was repeated sequentially for the 6 varnish dilutions, beginning with the 1:2 dilution added to each of

1 the 5 wells of column 2.

2 5. Samples were allowed to air dry at room temperature for 1
3 hour.

4 6. A second horseradish peroxidase (HRP) conjugated
5 monoclonal anti - *Listeria monocytogenes* antibody (MAb 832)
6 was diluted to a 1:4000 concentration in phosphate buffered
7 saline (PBS), pH 7.4.

8 7. Heat killed *Listeria monocytogenes* cells (antigen) were
9 added to the HRP conjugate solution at a concentration of 10^5
10 cells per mL.

11 8. 100 μ L of the antigen/conjugate solution, representing
12 10,000 *Listeria monocytogenes* cells, was added to each well
13 of the template and allowed to incubate for 1 hour at room
14 temperature.

15 9. The template was disassembled and the sheet of PE washed
16 briefly with UHF water to remove any excess conjugate.

17 10. The polyethylene sheet was placed in a 50 mL bath of TMB
18 substrate for peroxidase (available from Vector
19 Laboratories).

20 11. Color development was allowed to continue for 15 minutes
21 prior to removing the PE sheet from the substrate bath. The
22 reaction was stopped by rinsing the PE sheet with UHF water.

23 **Results:**

24 1. No color development was observed in columns 1 - 4.

1 2. Distinct color development was observed in each well of
2 columns 5 - 7.

3 3. Color could not be removed by the application and
4 subsequent lifting of adhesive tape.

5 Color development indicates that the biological activity
6 of the capture antibody applied to the PE surface is not
7 adversely affected by Water Gloss FDA Overprint Varnish
8 WVG001006. Alternatively, the absence of color development
9 in columns 1 - 4 (neat - 1:10 dilutions) indicates that a
10 threshold exists in the concentration of varnish applied to
11 the polyethylene surface. Binding is thus inhibited at
12 concentrations lower than 1:20. Furthermore, the inability
13 to remove color from the PE surface using adhesive tape
14 indicates that binding of the immunoglobulin to the PE
15 surface is stable and that leaching from the PE surface over
16 time will not occur.

17 All patents and publications mentioned in this
18 specification are indicative of the levels of those skilled
19 in the art to which the invention pertains. All patents and
20 publications are herein incorporated by reference to the same
21 extent as if each individual publication was specifically and
22 individually indicated to be incorporated by reference.

23 It is to be understood that while a certain form of the
24 invention is illustrated, it is not to be limited to the

1 specific form or arrangement herein described and shown. It
2 will be apparent to those skilled in the art that various
3 changes may be made without departing from the scope of the
4 invention and the invention is not to be considered limited
5 to what is shown and described in the specification and
6 drawings/figures.

7 One skilled in the art will readily appreciate that the
8 present invention is well adapted to carry out the objectives
9 and obtain the ends and advantages mentioned, as well as
10 those inherent therein. The embodiments, methods, procedures
11 and techniques described herein are presently representative
12 of the preferred embodiments, are intended to be exemplary
13 and are not intended as limitations on the scope. Changes
14 therein and other uses will occur to those skilled in the art
15 which are encompassed within the spirit of the invention and
16 are defined by the scope of the appended claims. Although
17 the invention has been described in connection with specific
18 preferred embodiments, it should be understood that the
19 invention as claimed should not be unduly limited to such
20 specific embodiments. Indeed, various modifications of the
21 described modes for carrying out the invention which are
22 obvious to those skilled in the art are intended to be within
23 the scope of the following claims.

24